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A NEW APPROACH FOR THE CONTROL OF COCKROACHES UTILIZING THE ENT--ETC(U)

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
	AD A118512	
4. TITLE (and Subtitle)	5. TYPE OF REPORT & PERIOD COVERED	
A New Approach for the Control of Cockroaches Utilizing the Entomophilic Nematode DD-136 in Conjunction with Attractants	Annual	
	6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s)	8. CONTRACT OR GRANT NUMBER(s)	
G. Mallory Boush	N00014-80C-0080	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	
University of Wisconsin-Madison Madison, WI 53706		
11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE	
Office of Naval Research 800 N. Quincy Street Arlington, Virginia 22217		
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	13. NUMBER OF PAGES	
	15. SECURITY CLASS. (of this report)	
	Unclassified	
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report)		
Distribution of this report is unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Block 16)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
Nematode Control Cockroach attractants Insect Growth Hormones		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
<p>The pathogenicity of two different entomogenous nematodes (<u>Neoaplectana carpocapsae</u> and <u>N. glaseri</u>) for the German cockroach (<u>Blattella germanica</u>) was investigated. Infective juveniles of each test species were placed in an infection chamber consisting of a standard petri dish with filter paper. At sufficient concentrations, the invasives of <u>N. carpocapsae</u> (DD-136) reduced roach populations significantly. However, the invasive of <u>N. glaseri</u> proved considerably less virulent. (Over)</p>		

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S/N 0102-LF-014-6601

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Visits to two feeding stations by individuals from a growing population of Blattella germanica were enumerated, aided by a closed circuit video system. The control station contained a pellet of a dry dog food diet only, while the other station contained, in addition, a sample of a food component being tested for its ability to attract. Observations were recorded for six hours per test, with each candidate substance tested once at each station. A positive response was tallied each time an individual entered the test station, and a negative response for each entry to the control station. Of the 30 substances tested, only 4 elicited 60 percent or more positive responses: oleyl alcohol, palmitic acid, finigreek seed alcohol extract, and elaidic acid methyl ester. These substances were re-evaluated using a two choice olfactometer. In these tests cockroaches could follow one of two air flows towards its origin after one flow had passed through a source of the candidate substance. All of these tests demonstrated independence.

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OFFICE OF NAVAL RESEARCH

Contract #N00014-80C-0080

Task No. NR 139-002

ANNUAL REPORT NO. 2

A New Approach for the Control of Cockroaches Utilizing the Entomophilic
Nematode DD-136 in Conjunction with Attractants

by

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August 1982

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This research was supported in part by the Office of Naval Research
Microbiology Program, Naval Biology Project under Contract N00014-80C-0080

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I. - Nematode Studies

Introduction:

Nematodes are multicellular animals that, like insects, have evolved to occupy nearly every biological niche. Although nematodes are generally considered as harmful organisms, attacking plants and parasitizing vertebrates; those species which attack invertebrates can play a significant role in limiting populations of agriculturally and medically important insect pests (Gaugler, 1981).

Although Poinar (1975) has listed nineteen families of nematodes that are facultative or obligative parasites of insects, it is the members of the genus Neoaplectana (Fam. Steinernematidae) that have shown the greatest potential as pest control agents. Of the hundreds of insect pests tested to date, nearly all have proved susceptible to and are killed by Neoaplectana species (Dutky 1974). The broad host range and high virulence of neoaplectanids make them attractive candidates for industrial development.

The most well studied of the neoaplectanid species is N. carpocapsae Weiser, which was first described from diseased codling moth larvae in 1954. Dutky and Hough (1955) later isolated a similar nematode which came to be known, after some disagreement as to its species status, as the DD-136 strain of Neoaplectana carpocapsae. Since then, other strains of N. carpocapsae have been recovered from various geographic locations. The most successful and encouraging attempts to control field populations of insects with nematodes have used strains of N. carpocapsae (Dutky, 1967; Poinar, 1979).

Another neoaplectanid, Neoaplectana glaseri, was first described in 1929 parasitizing Japanese beetle grubs in the eastern U.S. After a relatively short but illustrious history, the species fell into oblivion as far as biological control was concerned. Recently, however, interest has been

regenerated in this nematode because of the isolation of new field populations and the confirmation of the presence of an associated bacterium.

All of the neoaplectanids share a similar life cycle. The ensheathed third-stage juvenile is the invasive form which locates new hosts and initiates infection. The infective stages normally enter their hosts by way of the mouth, anus, or spiracles. After penetrating into the hemocoel, the nematodes release an associated bacterium from their intestinal lumen into the hemolymph. The bacterium multiplies rapidly, causing a fatal septicemia.

Poinar and Thomas (1965) first published a description of the entomopathogenic bacterium associated with N. carpocapsae, and named it Achromobacter nematophilus. Recently, a new genus, Xenorhabdus, has been created to accommodate the large, gram-negative, rod-shaped bacteria found intimately associated with entomogenous nematodes. To date, nine strains of Xenorhabdus, have been isolated and described, all of which are associated with members of either the Neoaplectana or Heterorhabditis.

The relationship between the nematode and its associated bacterium, Xenorhabdus nematophilus in the case of N. carpocapsae, is one of classic mutualism. The nematode protects the bacterium in nature and carries it into the hemocoel of a new host. In return, the bacterium, by furnishing some nutrient directly or indirectly, establishes conditions that are essential for nematode development and reproduction (Poinar and Thomas, 1966). The bacterium also represses cadaver colonization by other bacteria, thereby allowing the nematode to complete its life cycle without cadaver putrefaction.

Objectives:

The overall objective of this study is to determine whether entomogenous nematodes belonging to the genus Neoaplectana (or others, possibly

Heterorhabditis heliothidis) could be incorporated in some manner into a biological control program aimed at suppression of the German cockroach, Blattella germanica. Entomogenous nematodes potentially could provide a cheap, non-insecticidal alternative to the conventional methods used in attempts to control this pest. Since entomogenous nematodes (and associated bacterium) are already registered for use by the government, a detailed safety evaluation would be unnecessary.

More specific objectives (and current research) include:

- 1) the determination of the pathogenicity of different species of nematodes (particularly, DD-136 and N. glaseri) for the German cockroach, including:
 - a) dosage - mortality relationship
 - b) exposure time
- 2) to determine the ability of the nematodes to develop and reproduce within roach cadavers, producing second and third generation infectives capable of parasitizing new hosts.
- 3) to determine the ability of infectives to spread among members of a roach population.
- 4) to determine the potential of Xenorhabdus nematophilus as a pathogenic agent, and to elucidate other information concerning its role in nematode development.
- 5) the development of a trap and/or evaporetardant spray (utilizing nematodes) which can be field tested.

Materials and Methods

In order to determine the pathogenicity of N. carpocapsae (DD-136 strain) and N. glaseri for the German cockroach, the following experimental conditions were employed.

Infection chambers consisted of standard size (15 x 100 mm) plastic petri dishes lined with three sheets of Whatman's #1 filter paper (7 cm). Approximately 20 adult male roaches (2-3 weeks old) were added to each of ten dishes (for each nematode concentration). Varying concentrations of infective juveniles suspended in 3 ml .15% formalin were added to the infection dishes. (The volume of inoculum remained constant in all tests, the concentration/ml varied.) Controls were treated in the same manner except that the inoculum contained only formalin.

All infection dishes were incubated at room temperature. The dishes were checked at 24-hour intervals (for 5 days) for roach mortality. Dead roaches were examined for the presence of infective juveniles inside the hemocoel. In the case of the DD-136 strain, the hemolymph of a number of dead roaches was sampled for the presence of Xenorhabdus spp. This was accomplished by aseptically removing hemolymph from the antennae of surface-sterilized cadavers, and streaking it onto nutrient agar plates. The hemolymph of healthy roaches was also sampled as a control. The plates were incubated in the dark at 28°C.

Results and Discussion:

From our investigations, it is clear that the DD-136 strain of N. carpocapsae has the capacity to infect and kill German cockroaches under laboratory conditions. Even though the infection dishes provided optimum conditions, assuring host-parasite contact, the ability of the nematode-bacterium complex to cause roach mortality was impressive. With sufficiently high concentrations of DD-136, mortality rates reaching 100% were achieved in 72 hours. (See Table 1). Even at lower concentrations, significant mortality occurred, although the knockdown was not as rapid. Infective juveniles were readily observed in the hemocoel of dead roaches, especially in the abdominal

cavity, genitalia, head capsule, and legs. Attempts to isolate Xenorhabdus nematophilus from the hemolymph of infected roaches was successful in many cases. The hemolymph of normal, healthy roaches contained no microorganisms.

As a pathogen for roaches, N. glaseri was considerably less virulent than the DD-136 strain. At low doses, the effect of the invasives was negligible. Mortality rates did not exceed those of the controls. (See Table 2.) At higher doses, some deaths could be attributed to invasion by N. glaseri. Third-stage juveniles could again be observed in the hemocoel of many cadavers. Even at higher doses, though, the mortality rates were significantly lower than those achieved using comparable doses of the DD-136 strain. (See Figure 1.)

The relative inability of N. glaseri infectives to reduce roach populations could be due to several factors. The larger size of the invasives (1.06 mm compared to .547 mm for DD-136; Poinar, 1979) could be a disadvantage, making it more difficult to enter natural body openings. The greater surface area could render them more susceptible to desiccation. The infectives of N. glaseri may be less active than the DD-136 invasives when seeking a new host.

The ability of the DD-136 nematode to kill cockroaches has been clearly demonstrated. Since this nematode is significantly more virulent than N. glaseri, further investigations will concentrate mainly on the DD-136 strain. If the potential of this nematode can be developed, so that it can be used on a practical basis to manage roach populations, it will provide an attractive alternative to the conventional methods used to combat this insect pest.

Table 1. Percent mortality of roaches infected with N. carpocapsae (DD-136 strain)

Dosage/ml	N	Time interval (hours)				
		24	48	72	96	120
5,000	193	4.1	48.7	76.1	91.7	100.0
10,000	198	15.1	42.9	59.5	80.8	89.3
20,000	191	23.0	70.6	97.9	100.0	—
40,000	178	69.1	96.0	100.0	—	—
Control	198	0.0	3.5	10.6	21.2	40.9

N = no. roaches tested

Table 2. Percent mortality of roaches infected with N. glaseri

Dosage/ml	N	Time interval (hours)				
		24	48	72	96	120
5,000	180	0.0	5.5	7.2	8.8	12.2
10,000	197	21.8	35.0	47.7	54.8	60.4
20,000	173	4.6	13.8	23.1	36.9	44.5

N = No. roaches tested

Note: Because of the larger size of N. glaseri, the concentration/ml cannot exceed 20,000 infectives.

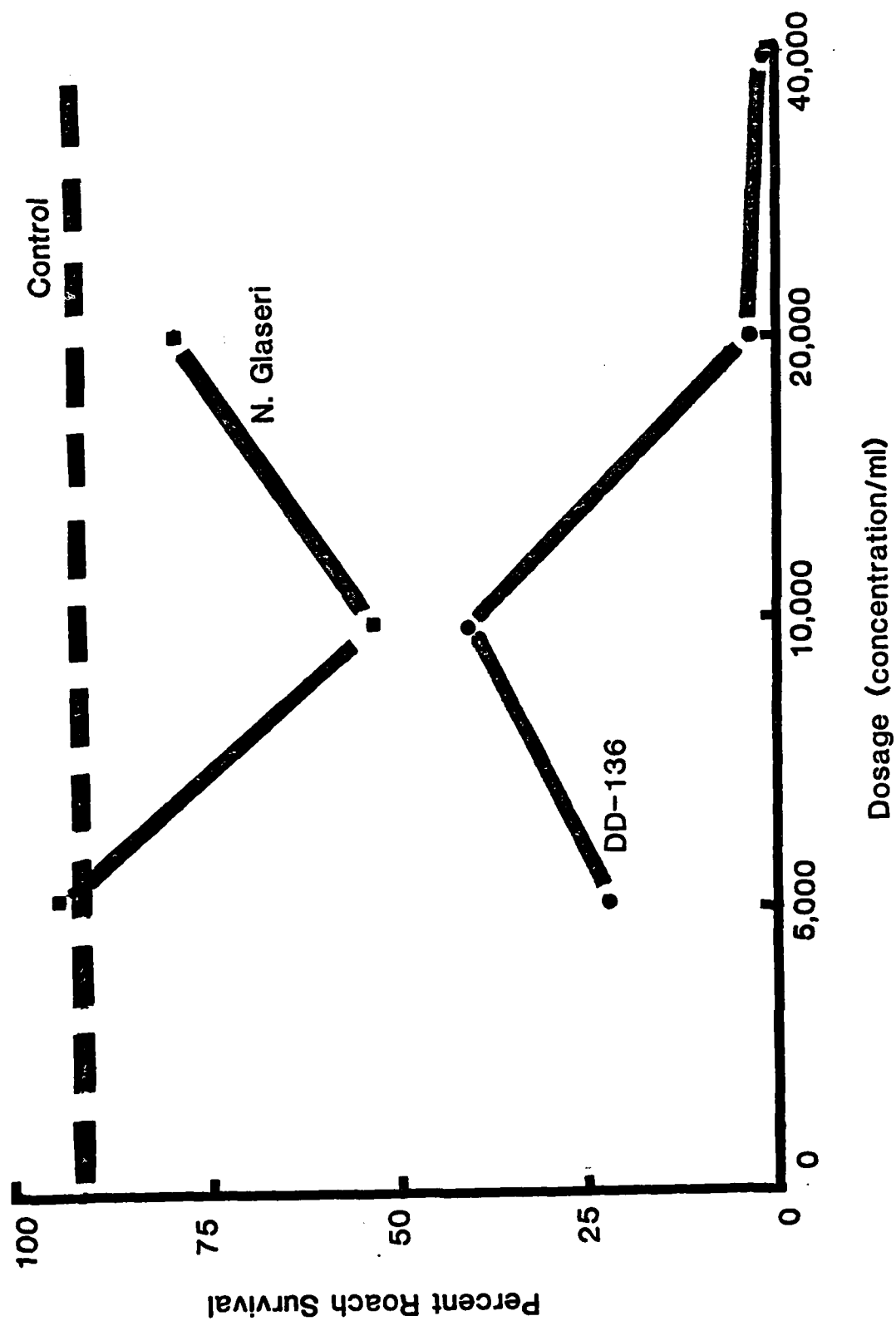


Figure 1: Percent Roach Survival after 72 hrs.

References:

1. Bedding, R. A. 1976. New methods increase the feasibility of using Neoaplectana spp. (Nematoda) for the control of insect pests. Proceedings from the First International Colloquium on Invertebrate Pathology, Kingston, Canada.
2. Dutky, S. R. 1967. An appraisal of the DD-136 nematode for the control of insect populations and some biochemical aspects of its host -- parasite relationships. Proc. Joint U.S.-Japan Seminar on Microbiol Control of Insect Pests, Fukuoka, pp. 139-140.
3. Dutky, S. R. 1974. Nematode parasites. Proceedings of the Summer Institute on Biological Control of Plant Insects and Diseases. U.P. Miss. 576-590.
4. Gaugler, R. R. 1981. Biological control potential of Neoaplectanid nematodes. J. Nematology. 13: 241-249.
5. Poinar, G. O., Jr. and G. M. Thomas. 1965. A new bacterium, Achromobacter nematophilus, sp. nov., (Achromobacteriaceae: Eubacteriales) associated with a nematode. Int. Bull. Bact. Nomen. and Tax. 15: 249-252.
6. Poinar, G. O., Jr., and G. M. Thomas. 1966. Significance of Achromobacter nematophilus, Poinar and Thomas (Achromobacteriaceae: Eubacteriales) in the development of the nematode, DD-136 (Neoaplectana sp. Steiner - nematidae) Parasitology 56: 385-390.
7. Poinar, G. O., Jr. 1979. Nematodes for biological control of insects. Boca Raton, Florida : CRC Press.
8. Thomas, G. M. and G. O. Poinar, Jr. 1979. Xenorhabdus gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. Int. J. Syst. Bact. 29: 352-360.

II. - Food Attractant Study

Introduction

The ability to attract roaches from a distance is important in their control, either for reducing numbers directly, for directing individuals to poisons or pathogens, or for monitoring populations. Volatile food components and products of degradation have been of interest as insect attractants since the realization that insects use these compounds in locating food (Dethier 1947). Pheromone assisted trapping and baiting has not produced the control expected (Silverstein 1981) and the potential of pheromones for use in controlling the German cockroach is further reduced in that they are perceived by touch (Persoons and Ritter 1979). Tsuji (1965) first characterized compounds attractive to roaches from fractions of rice bran, a preferred food, and food components remain the principle commercial attractants (Barak, et al. 1977).

Several types of compounds have long been suspected of aiding insects in the location of food. Many are products of the degradation of proteins and fats, while others are microbial components or products of fermentation (Dethier 1947; Tsuji 1965, 1966). Prevalent among the compounds named by these authors are fatty acids, esters, and alcohols. Products of animal decay such as amines, indoles, and sulfur compounds are generally less attractive (Dethier 1947) and the foods preferred by most roaches contain carbohydrates and limited proteins (Rau 1945; Cornwell 1968).

Given the predominance of fatty acids in the aggregation pheromone of the German cockroach, it has been suggested that their perception of these chemicals is advanced compared to their perception of other sorts of compounds

(Ritter and Persoons 1975). Many fatty acids, esters, and related substances have been tested for their ability to attract German cockroaches using a variety of methods (Tsuji 1965, 1966; Sugawara, et al. 1975; Reiersen and Rust 1977). Carbohydrates and sugars have been demonstrated to stimulate feeding, but volatility is low and hence, attraction doubtful (Dethier, et al. 1960; Tsuji 1965). Other more specific foods and extracts are suggested by numerous sources, though attempts to identify the specific compounds that are attractive are infrequent.

Numerous methods for measuring attraction are available in the literature. Attraction by a substance generally has been measured by presenting to test animals either a choice of the test substance versus a blank control, or the test substance plus a carbohydrate (non volatile) food source versus the food source alone. Tsuji (1966) generated an index of attraction from visual estimates of the degree to which roaches chewed paper impregnated with an 'attractant' plus sugar versus paper impregnated with sugar alone. Though the method was elegant, the index generated was discontinuous. Continuous measures of attraction have been provided by open system olfactometry¹ (Burkholder 1970), closed system olfactometry (Ebeling, et al. 1966; Rust and Reiersen 1977), and trapping (Sugawara, et al. 1975; Reiersen and Rust 1977).

Of these numerous tests, none has been performed uniformly for all of the many substances suggested in the literature. Apart from the measurements, test insects have varied in the degree to which they were starved, and tests were often performed against 'sterile' monotonous backgrounds. The purpose of this study was to review the many compounds suggested by the literature as being attractive to the German cockroach, and test them uniformly with

abundant food and a background of food odors. To eliminate the setup time associated with olfactometers while providing a continuous measure of attraction, foraging behavior in a population of Blattella germanica was observed from videotape at 3X normal speed. Members of the population could choose to enter one of two feeding stations, one impregnated with a test substance while the other was not.

Methods

A population of Blattella germanica² was housed at the center of a stainless steel circular arena 1.0 meter across and 0.15 meter deep and painted with a flat grey enamel that contrasted with the roaches, while not presenting a glare to the video camera. The inside of the wall was greased with a 1:1 (volume to volume) mixture of petroleum jelly and parafin oil to discourage climbing. A final barrier to escape was a coating of Tanglefoot® at the lip of the arena. To house the colony, an harborage was made from 1/8 inch masonite using ten square pieces 10 cm. on a side separated by square pieces 2 cm. x 2 cm. Watering stations were made from 8 oz. medicine bottles with a wick and plug made from 6 inch dental rolls. Feeding stations consisted of 1/8 inch thick circular glass plates 88 mm. in diameter, with one nugget of Purina® Dog Chow at the center. Two each of watering and feeding stations were placed in the arena, all equidistant from the harborage and wall, with watering stations at 0° and 180° and feeding stations at 90° and 270°. The colony was growing, and was culled whenever it was too large to be contained within the harborage. At any time, the colony contained between 300 and 700 individuals.

Most test substances were available commercially. Cyclohexyl compounds

were prepared as in Sugawara, et al. (1975). Samples were attached to feeding stations using a 2 cm. segment cut from 6 inch dental rolls, and impregnated one hour before filming with a solution of the test substance for the test feeding station or the solvent only for a control. Clean plates were used at the beginning of each run, and a fresh nugget of dog food, of similar mass, used each day of testing. A total fatty acid assay using the method described by Ho (1970) was performed to ensure that the compounds tested were not overwhelmingly present in the dog food.

All experiments were carried out under diffuse light in a secluded room at off hours to remove the influence of lab activity. Recording began at 5:00 - 6:00 P.M., with the test substance on one side of the arena. After six hours of recording, a second run was started using the same substance at the opposite side of the arena. Results were monitored at 3X normal speed on videotape. A response was tallied for a feeding station when an individual stepped up on to the glass plate. Individuals leaving a plate were tallied again only if they disappeared from view or moved to the harborage or a water station.

Independence of feeding between the two feeding stations was demonstrated using two plates with food only. The chi-squared (X^2) statistic was used to test for results significantly different from independence. Substances were ranked for attractiveness by the average proportion of responses in their favor.

Substances eliciting the greatest responses in the arena underwent testing in a two-choice open system olfactometer. The apparatus is a modified version of Burkholder's (1970) multiple choice olfactometer. The modified device consists of two glass plates with a brass spacer and gaskets between

them which collectively enclose a 'Y' shaped cavity. A vacuum line draws air at 0.5 litres per minute from the bottom leg of the 'Y'. Air enters the chamber at the ends of the other two legs. Incoming air was dessicated and charcoal filtered, and for one of the upper legs, air was exposed to the test substance by passing over a 2 cm segment of dental roll that had been impregnated with the chemical. Roaches numbering 150 to 200 were rendered unconscious with CO₂ gas and introduced to the bottom leg of the 'Y'. The apparatus was covered with black felt to prevent disturbance by lab activity. After 25 minutes, the chamber was flooded with CO₂ to anesthetize the roaches. Similar limits are drawn on each of the upper legs of the 'Y', and counts are made for each side within these limits. The apparatus was thoroughly cleaned with acetone between runs to remove any contaminants acquired during the previous run.

The two sides of the olfactometer were tested for independence five times initially, and once at the beginning of each operating day. Five tests of each 'attractant' were performed on each side of the olfactometer, yielding proportions of responses in favor of the chemical. Tests were run in four series, and independence for each series sometimes deviated from the usual 1:1 expectation. The 't distribution' was used to test for departures from independence.

Results

A review of the literature provided a list of food constituents that had been implicated as roach attractants.

Results of the arena experiment are analyzed in Table 3. Rank was determined by an average of the proportion of responses in favor of the substance. The chi-squared (X^2) value was not used in ranking since X^2 for a

given proportion will increase with sample size and the sample size varied considerably.

Confidence limits may be estimated for proportions taken from single samples (Dixon & Massey 1969) but the method is graphical and thus crude. A simple way to determine sufficiency of samples is to observe variation in data as it accumulates to determine a point where adding further observations will make little change in the estimate (often referred to as a 'running mean'). In most cases a sample of 200 responses was sufficient to produce results consistent in magnitude and direction. Aberrant results were lost to sample size after 300 responses. Very few of the samples are smaller than this.

Several of the test substances appeared to attract roaches in significant numbers. Only four substances elicited 60% or more of the total responses: oleyl alcohol, palmitic acid, the alcohol extract of finigreek seed, and elaidic acid methyl ester. Responses to oleyl alcohol and oleic acid were equal in magnitude yet opposite in direction, a result unexpected considering their similarity in structure. The trans isomer of oleic acid, elaidic acid, produced results near independence, while the methyl ester of this acid was one of the few to draw more than 60 percent of responses.

Tests using the open system olfactometer produced results close to independence, unlike tests in the arena. Normality was assumed for the distribution of proportions around the mean; since all values were between 0.30 and 0.70 there will be no inherent kurtosis (see Sokal & Rohlf 1981, section 13.10). Only one set of results approached significance: that for palmitic acid. All others strongly demonstrated independence.

Table 2. Summary of observations in the arena. Under 'Total' proportion (R) represents an average (weighed by sample size) from both sides of the arena. N is Sample size, R is fraction of responses on favor of substance tested, and P is the likelihood by chance of a χ^2 greater than or equal to the observed.

Side 1		Side 2						
Rank	Test Substance	N	R	P<	N	R	P<	R
1	Oleyl alcohol	400	0.718	0.0001	400	0.615	0.001	0.666
2	Palmitic acid	212	0.623	0.005	222	0.622	0.005	0.622
3	Finigreek seed alcohol extract	313	0.601	0.005	411	0.594	0.005	0.598
4	Elaidic acid	140	0.607	0.015	355	0.561	0.025	0.584
5	methyl ester							
5	Nonadecanol	400	0.593	0.005	400	0.570	0.005	0.581
6	Tricaprin	400	0.558	0.025	400	0.583	0.005	0.570
7	Galactose	262	0.561	0.05	236	0.576	0.015	0.568
8	Brown sugar	900	0.566	0.005	886	0.544	0.01	0.555
9	Maltose	304	0.549	0.10	261	0.559	0.10	0.554
10	Cetyl alcohol	330	0.561	0.03	446	0.536	--	0.548
11	Arabinose	115	0.626	0.01	107	0.449	--	0.537
12	Glycerol	669	0.529	--	816	0.526	--	0.528
13	Sucrose (refined)	618	0.534	--	738	0.515	--	0.524
14	Banana flavor	556	0.520	--	738	0.515	--	0.524
15	Sorbitol	788	0.513	--	719	0.530	--	0.522
16	Elaidic acid	265	0.551	--	125	0.464	--	0.515
17	Cyclohexylacetic acid n-propyl ester	563	0.510	--	513	0.511	--	0.510
18	Lauric acid	437	0.508	--	349	0.499	--	0.504
19	Palmitic acid methyl ester	164	0.470	--	364	0.525	--	0.498
20	Stearic acid	774	0.481	--	511	0.505	--	0.493
21	Pentanoic acid cyclohexyl ester	647	0.454	0.025	666	0.520	--	0.487
22	Tripalmitin	400	0.525	--	400	0.445	0.04	0.485
23	Caproic acid	287	0.505	--	395	0.458	0.10	0.482
24	Myristic acid	302	0.487	--	286	0.455	--	0.471
25	Artificial maple flavor	172	0.477	--	358	0.458	--	0.468
26	Palmitic acid ethyl ester	400	0.443	0.025	400	0.480	--	0.461
27	Caprylic acid	367	0.458	--	471	0.461	0.10	0.460
28	Myristic acid methyl ester	470	0.438	0.01	492	0.455	0.05	0.447
29	Oleic acid	342	0.427	0.01	520	0.454	0.05	0.441
30	Capric acid	683	0.399	0.001	503	0.459	0.10	0.429

Discussion

The results of this study differ to a great extent from those in the literature, and the results from the olfactometer differ considerably from those of the arena. These discrepancies begin to make sense in the light of the roaches' own food interests; that is asking how these insects should choose among odors or combinations of odors if they could pick and choose their own diet. Roaches prefer diets that are high in carbohydrates with minimal fats and proteins (Cornwell 1968). Breads seem to be a preferred food item (Rau 1945; Reiersen and Rust 1977), and the dregs of a beer bottle has more attractive power than anything tested in the laboratory (personal observation). Animal proteins draw almost no response (Rau 1945).

The individual food components that were tested in this study may carry a great deal of information about food content. To find foods that comprise their preferred diet, roaches may choose among the many volatile compounds present in the air that will lead them to those foods. The bulk of this discussion will consist in a consideration of the information that might be conveyed by these substances, either alone or in combination with other foods.

The cyclohexyl compounds produced results quite close to independence in the arena experiment. The 'ten fold' increase in response³ (Sugawara, et al. 1975) was not observable. The failure to increase attraction to a food source in our experiment was matched by tests comparing them against real foods and food extracts (Reiersen and Rust 1977). Under stringent conditions, these unnatural esters might allude to something edible, but in a kitchen where there are numerous carbohydrate sources, these unfamiliar compounds may have no appeal.

Substances commonly found in the foods that roaches seek are aliphatic acids, esters, alcohols, sugars, and carbohydrates (Tsuji 1965). Fatty acids

that were highly favored in Tsuji's (1966) study all produced results indicating independence or repellency, with the exception of palmitic acid. According to Dethier (1947) fatty acids derive from the breakdown of fats, and even more important, from the breakdown of proteins. The dog chow fed these roaches is already high in protein for these insects, and our analysis of total free fatty acids yielded an estimate of 1.12 percent. An average of 0.014 grams of undertermined fatty acids were present per nugget of dog food (average wt. 1.25 gm.). Though our addition of another 0.008 grams was not overwhelmed by the acid already present, the addition may have indicated an even higher protein content to the foraging roaches. Indeed there is a positive relationship, although not strong, of the percent of responses drawn by a fatty acid in the arena with its molecular weight, an indicator of decreasing volatility ($r^2 = .193$).

There is no apparent reason for the responses produced by esters, though they were quite different from tests in prior works (Tsuji 1966; Sugawara, et al. 1975). Most were ranked as neutral or repellent, with one notable exception: that of elaidic acid methyl ester. However, only four aliphatic esters were tested so any attempt to find a pattern is meaningless.

All alcohols tested were attractive. All but glycerol produced significant deviations from independence, and oleyl alcohol tops the list of attractants. Unexpected was the vast difference between oleyl alcohol and oleic acid, which have the same structure except for the terminal groups. This difference may carry a great deal of information about origin, since heavy alcohols are likely to come from fungal breakdown of carbohydrates (Dethier 1947) while fatty acids indicate proteins.

Sugars, though less attractive than alcohols, all elicited more than 50 percent of responses. This result was unexpected, since these substances were assumed to be non-volatile. With the exception of galactose, the order of attractiveness matches perfectly that from the work of Tsuji (1965). Brown sugar tops the list of sugars, while refined sucrose is near the bottom. Though the former had not been included in any previous studies, the impurities that make brown sugar different from refined sucrose apparently contribute a great deal to attraction.

One substance atop the list of attractants, an extract of finigreek seed, does not fit into any such pattern of explanation. Its striking ability to attract roaches has apparently led to some use in the control industry.

Re-evaluation of the best attractants using the olfactometer found them close to neutral in attractive ability. At least two hypotheses could explain this. The first is that a 'monotonous' odor will not bring unstarved roaches out of their way to eat. Another possibility (suggested by Ebeling and Reiersen 1974) is that roaches cannot orient themselves toward an odor over more than a small distance⁴. Further studies might reveal differences in the response of starved and unstarved individuals. Perhaps starved individuals may orient towards a monotonous odor. This would throw out the possibility of their being unable to orient towards an odor for any extended period of time.

Food attractants may prove especially valuable in monitoring populations and effectiveness of control efforts, masking pesticide repellancy, as well as luring cockroaches to toxicants or pathogens. A lot may rest not only on the identification of attractive compounds, but on the identification of attractive mixtures and the circumstances under which they will be used. Single compounds may never be able to compete with the foods from which they are isolated, especially when they have been trodden into the cracks of a

kitchen floor. Serious consideration should be given the use of such attractants with traps that present the appearance of a good harborage.

References

- Barak, A.J., M. Shinkle and W.E. Burkholder. 1977. Using attractant traps to help detect and control cockroaches. *Pest Control* 45(10) 14-20.
- Burkholder, W.E. 1970. Pheromone research with stored-product Coleoptera. In: *Control of insect behavior by natural products*. Academic Press, Inc., N.Y., NY.
- Cornwell, P.B. 1968. *The Cockroach*. Volume 1. Hutchinson & Co., Ltd., London, UK.
- Dethier, V.G. 1947. *Chemical insect attractants and repellents*. Blakiston, Philadelphia, PA.
- Dethier, V.G., L.B. Browne and C.N. Smith. 1960. The designation of chemicals in terms of the responses they elicit from insects. *Jour. Econ. Entomol.* 53:134-136.
- Dixon, W.J. and F.J. Massey. 1969. *Introduction to statistical analysis*. McGraw-Hill Book Co., N.Y., NY 638 pp.
- Ebell, W., R.W. Wagner and D.A. Reiersen. 1966. Influence of repellency on the efficacy of Blatticides. I. Learned modification of behavior of the German cockroach. *Jour. Econ. Entomol.* 59:1374-1388.
- Ebeling, W. and D.A. Reiersen. 1974. Bait trapping silverfish, cockroaches and earwigs. *Pest Control* 42(4) 24, 36-39.
- Ho, R.J. 1970. Radiochemical assay of long chain fatty acids using ^{63}Ni as tracer. *Analyt. Biochem.* 36:105-113.
- Noguchi, K. 1961. Cockroaches in a typewriting room. *Jap. Jour. Sanit. Zool.* 12:118.

- Ogata, K. and M. Mihara, 1962. Observations on the so called "butter trap" in trapping the German cockroach, Blattella germanica Linne. Jap. Jour. Sanit. Zool. 13: 2 62-267.
- Persoons, C.J. and F.J. Ritter. 1979. 'Pheromones in Cockroaches.' In: Chemical ecology: odour communications in animals. Elsevier/North-Holland Biomedical Press, Amsterdam, Netherlands.
- Rau, P. 1945. Food preferences of the cockroach, Blatta orientalis Linn. Entomol. News 56(10) 276-278.
- Reierson, D.A. and M.K. Rust. 1977. Trapping, flushing, counting German roaches. Pest Control 45(10) 40-44.
- Rust, M.K. and D.A. Reierson. 1977. Increasing blatticidal efficacy with aggregation pheromone. Jour. Econ. Entomol. 70:693-696.
- Silverstein, R.M. 1981. Pheromones: Background and potential for use in insect control. Science 213:1326-1332.
- Sokal, R.R. and F.J. Rohlf. 1981. Biometry, 2nd ed., W.H. Freeman and Company, San Francisco, CA 859 p.
- Sugawara, R., S. Kurihara and T. Muto. 1975. Attraction of the German cockroach to cyclohexyl alkanoates and n-alkyl cyclohexaneacetates. Jour. Insect. Physiol. 21:957-964.
- Tsuji, H. 1965. Studies on the behaviour pattern of feeding of three species of cockroaches, Blattella germanica (L.), Periplaneta americana L., and P. fuliginosa S., with special reference to their responses to some constituents of rice bran and some carbohydrates. Jap. Jour. Sanit. Zool. 16:255-262.
- Tsuji, H. 1966. Attractive and feeding stimulative effect of some fatty acids and related compounds on three species of cockroaches. Jap. Jour. Sanit. Zool. 17:89-97.

Tsuji, H. and S. Ono. 1969. Laboratory evaluation of several bait factors against the German cockroach. Jap. Jour. Sanit. Zool. 20:240-247.

Footnotes

¹Many olfactometers can keep the test animals separate from the test substance source, eliminating compounds perceived only by touch.

²A strain of B. germanica, CSMA-1, was obtained from the Raltech Corporation, Madison, WI.

³No mention was made by this author of the degree to which test insects were starved, or of any food source used in conjunction with the tests.

⁴This may well be true for Blattella germanica, as its pheromones are all sensed by touch (Ritter and Persoons 1975). Ebeling and Reiersen (1974) suggest this for all paurometabolous insects, but the response of Periplaneta americana to its aggregation pheromone is clearly olfactory (Ritter and Persoons 1975).

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